II Symposium

PORTUGUESE GLIAL NETWORK

GLIAL CELLS:
MUCH MORE THAN GLUE

Abstract book

May 24, 2017

Life and Health Sciences Research Institute (ICVS)
School of Medicine, University of Minho
Braga, Portugal
PROGRAMME

Location: School of Medicine – ICVS, Ground floor Seminar A0.02

WEDNESDAY, MAY 24th 2017

9:00-10:00 Reception of the participants at ICVS/MED hall

10:00-10:50 OLYMPUS Keynote lecture
Frank Kirchhoff (U. Saarland, Germany) - Specializations of astrocytes and oligodendrocytes determine brain function

10:50-11:00 Coffee break

11:00-13:00 MCS Session I – Neuronal and Glial Physiology

João Relvas (I3S, Porto) - Regulation of glial homeostasis
Adelaide Fernandes (iMed.UL, Lisboa) - Glia interplay during demyelination
Sandra Vaz (iMM, Lisboa) - On the role of astrocytes in synaptic plasticity
Camila Portugal (I3S, Porto) - Sodium Vitamin C co-Transporter-2 (SVCT2): A critical molecule for microglial physiology
Ligia Tavares (I3S, Porto) - The influence of the niche fitness in glia (over)migration

13:00-14:00 Lunch

14:00-15:45 NOLDUS Session II – Glial Dysfunction and Modulation in CNS Disorders

Dora Brites (iMed.UL, Lisbon) - Microglia phenotypic heterogeneity and vesicular trafficking in ALS disease
António Salgado (ICVS, Braga) - Extracellular Matrix-like Hydrogels and Stem Cells Secretome in CNS Regenerative Medicine
Ana G Mestre (U. Algarve, Faro) - Electrical detection of extra-cellular long-lasting potentials in astrocytes using polymer based devices
Rita Gaspar (IBILI/FMUC, Coimbra) - Modulation of microglia morphology by adenosine A2A receptors: impact for anxiety and cognition
Cláudia Filipa Afonso (FMUL, Lisboa) - Rat oligodendrogenesis is modulated by adenosine A2A receptors in vivo

15:45-16:00 Coffee break

16:00-17:45 VIEWPOINT Session III – Neuroinflammation

Francisco Ambrósio (IBILI, Coimbra) - Treatment of retinal degenerative diseases: targeting neuroinflammation?
Teresa Summavielle (I3S, Porto) - New insights on psychostimulant regulation of microglia activation
Andrea Cruz (INL, Braga) - Identification of actomyosin-based biomarkers relevant for microglia activation
Marta Barbosa (iMed-UL, Lisboa) - Recovery of miR-146a levels through overexpression reverts astrocyte reactivity in cells from ALS mice and modulate astrocytic exosome cargo

Renato Socodato (I3S, Porto) - Microglia homeostasis requires RhoA and its conditional ablation leads to phenotypes commonly associated with neurological disorders

17:45 Closing remarks

WE THANK YOUR KIND SUPPORT:

Sociedade Portuguesa de Neurociências
OLYMPUS Keynote lecture

Frank Kirchhoff

**Specializations of astrocytes and oligodendrocytes determine brain function**

Frank Kirchhoff

**Molecular Physiology, Center for Integrative Physiology and Molecular Medicine (CIPMM), University of Saarland, Homburg, Germany**

The human brain is an extraordinary complex structure, consisting of about 80 billion neurones, connected by numerous synapses, and of an equal number of neuroglial cells. Each second, around $10^{14}$ “arithmetic” operations are conducted, which are the basis for processes ranging from simple regulatory activity to sophisticated learning, memory and cognition. To fulfil all the different tasks related to information input, signal processing and output, different brain regions, and the neurons within, are highly specialized and can be functionally divided into a multitude of subpopulations.

However, recent research has revealed that about half of the brain consists of different classes of neuroglial cells. The main macroglial cell types of the brain, astrocytes, oligodendrocytes, radial glia and NG2 cells, also execute numerous essential functions. Astrocytes detect and modulate neuronal activity thereby affecting neuronal performance, regulate blood flow, take up nutrients and supply neurones with energy metabolites and, indirectly, with oxygen. Oligodendrocytes enwrap neuronal axons with lipid-rich lamellae to enhance action potential propagation. Radial glia or NG2 cells can serve as neural stem cells, generating new neurones or oligodendrocytes, respectively, in the developing and the adult brain.

In my presentation, I will highlight some recent observations how different glial cells contribute to brain function in health and disease using genetically modified mice in combination with two-photon imaging in vivo.
Regulation of glial homeostasis

João Relvas (1,2)

(1) Instituto de Investigação e Inovação em Saúde (I3S), Universidade do Porto, Porto, Portugal; (2) Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Porto, Portugal

Only very recently the scientific community has recognized microglia’s primary contribution for CNS homeostasis. Not only microglia are involved in physiological synaptic pruning during development and adulthood, but also produce a plethora of chemical mediators that can affect synaptic plasticity and consequently basic neurological functions, including memory and learning. Accordingly, several microglia-related genes have been implicated in brain disease triggering mechanisms, supporting the novel and emergent notion of “microgliopathy” in which microglia dysfunction can itself trigger brain disorder. The rising of microglia as a central player in CNS homeostasis has important implications for the understanding, prevention and treatment of brain disease. Consequently, to elucidate molecular mechanisms underlying microglia function is now an urgent need. In my presentation I will summarise our latest results concerning the regulation of microglia homeostasis by the Rho family of GTPases. We show that microglia-specific ablation of the small GTPase RhoA disrupts the homeostasis of mouse adult brain resident microglia leading to neuroinflammation, astrocytosis, impairment of long-term potentiation (LTP) and cognitive deficits.
**Glia interplay during demyelination**

Andréia Barateiro (1), Gisela Santos (1), Vera Afonso (1), Carla Ferreira (1), Jack van Horssen (2), Dora Brites (1,3), João J. Cerqueira (4), Adelaide Fernandes (1,3)

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One of the hallmarks of demyelinating disorders, including Multiple Sclerosis (MS), is neuroinflammation, being considered an important target to enhance remyelination. During demyelination, activation of astrocytes and microglia contribute to the inflammatory milieu that affects not only oligodendrocyte survival but also oligodendrocyte progenitor cell recruitment for de novo myelination. We recently showed that the pro-inflammatory molecule S100B is highly increased in the cerebrospinal fluid and serum of MS patients at diagnosis. Additionally, immunohistological analysis of post-mortem brain slides of MS patients revealed a higher expression of S100B in active and chronic MS lesions by astrocytes, while its receptor for advanced glycation endproducts (RAGE) was markedly expressed by macrophages/microglia in the vicinity of active MS lesions. Using an ex vivo demyelinating model, the cerebellar organotypic slice cultures treated with lysophosphatidylcholine (LPC), we detected a massive and prolonged expression of S100B upon demyelination, namely by astrocyte source. Next to assess the role of S100B in demyelination we used a neutralizing antibody against S100B or RAGE antagonist (RA) and observed that both compounds are able to reduce LPC-induced demyelination and loss of synaptic markers. Interestingly, also oligodendrocyte differentiation and maturation, delayed upon LPC demyelination, was rescued by RA. In parallel anti-S100B was also able to reduce the LPC-induced inflammatory milieu by abrogating the expression of first line cytokines TNF-α and IL-1β, as well as inflammasome-related IL-18, HMGB1 and NLRP3. Most attractively, microglia pro-inflammatory/M1 polarization observed upon LPC demyelination was rescued following anti-S100B treatment with reduction of specific markers MHC class II, iNOS and C/EBPα. Conversely, resolution of damage/M2 polarization also induced upon LPC demyelination was maintained as seen by continuous expression of Arginase 1 and FIZZ1. In accordance also microglia phagocytic ability, needed for myelin clearance following demyelination, was increased by anti-S100B treatment. Collectively, our results corroborate how important is glial interplay in demyelination by showing that astrocyte-derived S100B activates microglia and enhances the inflammatory milieu that affects oligodendrogenesis and subsequent remyelination. Further, since S100B plays a crucial role during demyelination, we may anticipate its potential therapeutic targeting to reduce damage and improve recovery in inflammatory-associated demyelinating disorders.

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Session I – Neuronal and Glial Physiology

Sandra Vaz

On the role of astrocytes in synaptic plasticity

Sandra H. Vaz (1,2), Haíssa de Castro (1,2), Andreia Cruz-Silva (1,2), João Jesus (1,2), Joana Gomes (1,2) and Ana M. Sebastião (1,2)

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Astrocytes are key cellular partners to neurons, playing an important role in multiple processes in the brain. Hippocampal long-term potentiation (LTP) is a sustained enhancement of excitatory synaptic strength believed to underlie learning and memory processes and recently has been described that astrocytes regulate synaptic transmission and play a role in shaping LTP [1]. Specifically, the release of gliotransmitters, such as glutamate, ATP, and D-serine likely alters the viability and functioning of newly formed connections. Other very important molecule for the modulation of LTP is the brain-derived neurotrophic factor (BDNF). BDNF is a growth factor involved in the development and maintenance of different neuronal population in the nervous system. Furthermore BDNF has a facilitatory action upon hippocampal LTP, being this action dependent on the adenosine A2A receptor (A2AR) activation [2, 3].

Thus is important to study the involvement of astrocytes upon the modulatory effect of BDNF upon LTP, the possible involvement of adenosine (and A2AR) on this process and the role of adenosine receptors activation on calcium signalling mediate by astrocytes.

These results will have a big impact in understanding the role of astrocytes in the CNS, but more importantly the role of astrocytes on the glial–neuron communication and on the effect of BDNF at synaptic levels.

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Sodium Vitamin C co-Transporter-2 (SVCT2): A critical molecule for microglial physiology

Camila C. Portugal (1), Renato Socodato (1), Teresa Canedo (1), Cátia M. Silva (1), Tânia Martins (3,4), Vivian S.M Coreixas (2,3), Erick C. Loiola (2,3), Burkhard Gess (5), Dominik Röhr (5,6), Ana R. Santiago (3,4), Peter Young (5), Richard D. Minshall (7), Roberto Paes-de-Carvalho (2), António F. Ambrósio (3,4), João B. Relvas (1)

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The sodium-vitamin C co-transporter 2 (SVCT2) specifically transports ascorbate, the reduced form of vitamin C, which works as a central reducing agent in the central nervous system (CNS). Here, we demonstrate that ascorbate uptake through SVCT2 is critical for microglia homeostasis. SVCT2 depletion and consequently reduction in ascorbate uptake, resulted in a proinflammatory phenotype, and was also required for microglia activation by different inflammatory stimuli. Preventing SVCT2 internalization directly or through its regulatory pathways prevented microglia activation, and might constitute an interesting therapeutic strategy to attenuate the deregulation of microglial activity in aging and disease.

Firstly, we characterized the SVCT2 expression and functioning in microglia and its behavior during proinflammatory stimulation. To explore the role of SVCT2 in the pro-inflammatory stimulation of microglia, we challenged Wistar rats retinas by ischemia-reperfusion injury or LPS intravitreous injections and evaluated the expression of SVCT2 by high-resolution confocal microscopy. We showed that both stimuli trigger a robust SVCT2 downregulation in retinal microglia.

To better understand the role of SVCT2 in microglia, we performed shRNA-mediated SVCT2 Knockdown and observed a pro-inflammatory signature in primary retinal microglia, with increased ROS production, nuclear translocation of NF-kB and increased pro-inflammatory cytokine production (measured by qRT-PCR and ELISA). Consistent with a critical role for SVCT2 in activating microglia in vitro, SVCT2+/− mice showed pronounced microgliosis both in the retina and cerebral cortex.

We also analyzed the signaling pathways involved in this process and showed (by biotinylation, immunoprecipitation and FRET) that c-Src phosphorylates caveolin-1 at Tyr14 leading to caveolin-1-mediated SVCT2 internalization and degradation in the lysosome. Finally, we showed that LPS-induced microglia activation was abolished by overexpressing either SVCT2 or caveolin-1 phosphodefective construct (Y14F). Overall, our data demonstrate an essential role for SVCT2 in controlling microglial physiology.
The influence of the niche fitness in glia (over)migration

Ligia Tavares, Andreia Correia, Marilia Santos, João B. Relvas, Paulo S. Pereira

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The nervous system is formed by neurons, which transmit information from cell to cell, and by glia, which supports and maintains a healthy and functional neuronal network. Examples of the importance of glial cells for the functioning of both the central nervous system (CNS) and the peripheral nervous system (PNS) can be found on the support of neuronal survival and as the immune cells for the CNS in addition to providing insulation and trophic support to neurons. In addition to neurons and glia the nervous system niche is important for the proper development of both glial cells and neurons being the trigger of some of the most invasive cancers. To address the importance of the fitness of neuronal progenitors for glial cell development we depleted Drosophila MYC (dMYC) protein in Drosophila retinal photoreceptor progenitors. MYC is one of the best-described proteins which mutations affect the “cellular fitness” at the level of cell growth and proliferation. In this study we show that Myc-depleted retinal progenitors induce glial cells increased proliferation, pMad activation and overmigration. This stress response is mediated by JNK activation and facilitated by MMP1 expression. We are currently addressing the mechanisms used by glial cells to monitor and respond to defective status in a developing nervous system, possibly in a homeostatic fashion.
Amyotrophic lateral sclerosis (ALS) is the most common and most aggressive form of adult motor neuron degeneration. Although initially thought to only derive from the selective loss of motor neurons, the pathogenic concept of non-cell-autonomous in ALS disease has come to the forefront for the contribution of glial cells, in particular microglia. However, the nature of microglial–neuronal interactions that lead to motor neuron degeneration remains elusive. Some Authors indicate that NF-kappaB activation is implicated in microglia induced motor neuron death and that the cells acquire a non-M1/M2 signature. Although several genetic mutations have been found, the mutant human SOD1G93A in NSC34 cells and the SOD1G93A mice are standard models for the evaluation of mechanisms, identification of targets and assessment of therapeutic effects. In this presentation we will report the profiling of miRNAs in the spinal cord, as well as in microglial cell line N9 exposed to exosomes from SOD1G93A NSC-34 motor neurons, and in primary microglia isolated from the SOD1G93A mice. Using the spinal cord of the SOD1G93A mice model we will show that the inflammatory profile changes from a depressed signature in the presymptomatic state to a proinflammatory status at the symptomatic phase. Upregulation of miRNA-155 and downregulation of MFG-E8 at both periods sustain their utility as targets for drug discovery and as biomarkers of the disease. In addition, we will further highlight that microglia isolated from the SOD1G93A mice spinal cord and cultured from 2 days and 16 days in vitro switch the phenotype from activated to a low responsive subtype, with increased expression of both calming and stressed miRNAs. Upregulation of miRNA-155 is again a sustained feature in both conditions. We next will address the role of exosomes released by motor neurons and their content in miR-124 on microglia activation. Recent evidence indicates that these cells release endosome-derived microvesicles termed exosomes, which are 50–100 nm in size and carry specific protein and RNA cargo. Such exosomes were suggested to be implicated in ALS dissemination by being involved in cell-to-cell spreading of toxic molecules and disease-associated pro-inflammatory cytokines and miRNAs. In this talk it will be addressed the question as to whether exosomes released by SOD1G93A NSC-34 motor neurons, and their cargo in miRNA, are responsible for microglia activation and how far the acquired phenotype signature recapitulates that of SOD1G93A mice at the symptomatic stage. Examination of the implicated inflammatory mediators and miRNAs in the ALS used models may yield information useful for translating results to clinical application.

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Session II – Glial Dysfunction and Modulation in CNS Disorders

António Salgado

Extracellular Matrix-like Hydrogels and Stem Cells Secretome in SCI Regenerative Medicine

António J. Salgado (1,2)

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The low regeneration potential of the central nervous system (CNS) represents a challenge for the development of new therapeutic strategies. Mesenchymal stem cells (MSCs) have been proposed as a possible therapeutic tool for CNS disorders, namely due to the beneficial actions of their secretome. Indeed, the latter possesses a broad range of neuroregulatory factors that promote an increase in neurogenesis, inhibition of apoptosis/glial scar, immunomodulation, angiogenesis, neuronal and glial cell survival, as well as relevant neuroprotective actions into different pathophysiological contexts. Considering their protective action in lesioned sites, MSCs’ secretome might also improve the integration of local progenitor cells in neuroregeneration processes, opening a door for their future use as therapeutical strategies in human clinical trials. In this sense the secretome of MSCs could represent an important vehicle for future CNS regenerative therapies. In the present talk the role of the secretome of MSCs, from different sources, on phenomena such as in vitro and in vivo neuronal/glial survival will be addressed. Additionally the possible applications of secretome based therapies (for Parkinson’s Diseases and Spinal Cord Injury regenerative medicine will also be discussed. Finally new trends on how to modulate the secretome MSCs will also be presented, particularly the use if hydrogels and bioreactors.
Electrical detection of extra-cellular long-lasting potentials in astrocytes using polymer based devices

Ana G. Mestre (1,2), Pedro Inácio (1,2), Sanaz Asgarifar (1,2), Inês M. Araújo (3,4,5), and Henrique L. Gomes (1,2)

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Long-lasting or slow electrical fluctuations are normally found in the extracellular milieu. These are caused by ions and charged molecules that can move from cell to cell by gap junctions. These fluctuations are steady or slowly changing gradients and they progress thousands of times more slowly than action potentials. This slow activity does not show spikes but smooth potentials that can change over a period of time from several seconds to minutes. Astrocytes are neural cells that generate this type of slow signals. Indeed, astrocytes cells exhibit a form of excitability that modulate synaptic activity. This process has been named “gliotransmission” introducing the notion that information processing is not a unique feature of neurons. Until now these slow ionic fluctuations have been recorded using optical techniques. Here we show that these electrical fluctuations can also be recorded using extracellular conducting polymer electrodes in cell cultures in a totally non-invasive way and over extended periods of time.

The detection limit of extracellular electrodes was brought down to the low nanovolt range. Bioelectrical signals with amplitudes of 150 nanovolts in a noise level of 20 nanovolts (peak-to-peak) were recorded. The strategy behind this ultra-high sensitivity is the use of high capacitive polymer based electrodes and observation windows below 10 Hz. The electrodes and the methodology are demonstrated by recording ultra-weak signals produced by primary cultures of astrocytes and in C6 cell line (astroglioma). Our electrical recordings are comparable with optical fluorescence recordings reported in literature.

We propose that polymer based devices are a powerful tool to unravel the dynamics of neuroglial ionic signalling.

Keywords: Astrocytes, cooperative bioelectrical activity, polymer-based electrodes.
Modulation of microglia morphology by adenosine A2A receptors: impact for anxiety and cognition

Rita Gaspar (1,2), Joana Mendes Duarte (1,2), Patricia Patricio (3,4), Carina Cunha (4), António Mateus-Pinheiro (3,4), Nuno Dinis Alves (3,4), Ana Rita Santos (4), Samira G. Ferreira (2,5), Vanessa Sardinha (4), João Filipe Oliveira (3,4), Nuno Sousa (3,4), Rodrigo A. Cunha (2,5,6), António F. Ambrósio (1,2,6), Ana João Rodrigues (3,4), Luísa Pinto (3,4), Catarina A. Gomes (1,2,6)

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Microglial cells are important for brain development. Their morphology is critical for brain functioning and undergoes profound remodeling in neuropsychiatric diseases. Previous work from our research team showing that anxiety associated with prenatal exposure to dexamethasone (DEX), a synthetic glucocorticoid (GC), correlates with gender-specific changes in microglia morphology in the medial pre-frontal cortex (mPFC), strengthens the role of these cells in the developmental genesis of anxiety. Moreover, the pharmacologic blockade of adenosine A2A receptor (A2AR), described as cognitive enhancer and modulator of microglia morphology and involved in the pathophysiology of anxiety was unable to treat prenatal DEX-induced anxiety in females, but improved cognition.

The goal of the present study was to investigate the effects of prenatal exposure to DEX on microglia morphology in the postnatal period and at adulthood (postnatal day, PND7 and PND90) in the dorsal hippocampus (dHIP). Besides that, we aim clarifying if this functional uncoupling between anxiety and cognition in females treated with the A2AR antagonist could be explained by a dual, brain-region specific effect of A2AR in the control of microglia morphology. Animals were prenatally exposed to saline or DEX (1 mg/kg) and treated with saline or a selective A2AR antagonist, SCH 58261 (0.1 mg/kg for 21 days before PND90). Morphometric analysis of microglia in females at PND7 and PND90 was performed by immunohistochemistry (myeloid marker, Iba-1) followed by manual reconstruction using the Neurolucida software and the functional coupling between mPFC and dHIP was assessed by in vivo electrophysiology. Prenatal exposure to DEX triggered short-term effects in the dHIP, namely a significant decrease in the length of ramifications, an effect selective to the more proximal processes. At adulthood, DEX triggered long-term effects in the dHIP, namely a hyper-ramification of microglial cell processes and reverted mPFC-dHIP desynchronization, in line with a cognitive enhancement. The chronic blockade of A2AR restored microglia morphology. These results contrast with observations in the mPFC, where DEX induced a long-lasting atrophy of microglia, not reverted by blocking A2AR, which did not ameliorate anxiety. Our data show that exposure to high GC levels during uterine development induces alterations in microglia morphology in a brain-region specific manner and increase the risk of cognitive deficits in association with altered synchronization between mPFC-dHip. These changes correlate with functional recovery by A2AR blockade correlated with the morphological recovery of microglia in the dHip, a brain region involved in memory consolidation.

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Oligodendrocytes (OLGs) are the cells responsible for myelinating the neuronal axons of the Central Nervous System (CNS). The emergence of the myelin sheath was an important event in vertebrate development, allowing the rapid propagation of action potentials while preserving axonal diameter and reducing metabolic costs of neuronal activity. During a demyelinating disorder, such as multiple sclerosis (MS), myelin disruption and oligodendrocyte death is observed. In MS, remyelination occurs with new oligodendrocytes being generated from brain parenchymal oligodendrocyte progenitor cells (OPCs) and a minority from neural stem cells present in the subventricular zone (SVZ) along the lateral ventricles. Interestingly, previous data from our group showed that adenosine A2A receptor (A2AR) activation stimulates SVZ oligodendrogenesis in the context of the neurosphere assay. Therefore, our aim was to evaluate the role of A2ARs in rat oligodendrogenesis in vivo.

A cannula was inserted in the right lateral ventricle of 6-week old Wistar rats and connected to an osmotic minipump, from which the A2AR agonist (CGS21680 100 nM) or the vehicle was delivered continuously for 28 days. Cell proliferation was evaluated with two intraperitoneal injections of BrdU at the end of drug administration, separated by 2-hour intervals. Cell migration was studied by injecting animals with BrdU twice a day at 12-hour intervals in the first three days of treatment. Immunohistochemical processing was performed for Olig2 and BrdU.

Our results unexpectedly showed that using the proliferation protocol, activation of A2ARs in the SVZ decreases the proliferation of OPCs, leading to a reduction in the percentage of double-labeled cells for BrdU and Olig2 per volume (control: 100.0%±16.0, CGS21680: 34.0%±8.6, N=3, *p=0.0222) and per total BrdU-positive cells (control: 8.3%±1.2, CGS21680: 4.2%±1.2, N=3, ns, p=0.0715). Furthermore, the percentage of BrdU-positive cells per volume of SVZ is also diminished following A2AR activation (control: 100%±13.3, CGS21680: 69.8±7.9, N=3, ns, p=0.1236). Most of these double-labeled Olig2 and BrdU cells migrate to the OB via the rostral migratory stream (RMS). However, using the differentiation protocol, we observed that A2AR activation slows the migration of Olig2-positive cells, since a lower percentage of double-labeled cells, both per volume (control: 100.0%±8.7, CGS21680: 22.5%±9.8, N=3, **p=0.0060) and per total BrdU-positive cells (control: 17.2%±2.6, CGS21680: 5.0%±0.5, N=3, *p=0.0105) is observed in the RMS following administration with CGS21680.

A2AR activation appears to negatively modulate SVZ oligodendrogenesis in vivo. However, further immunohistochemical analysis of cells in the later stages of oligodendrocyte development will be necessary to determine the precise role of A2ARs in this process.

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Session III – Neuroinflammation

Francisco Ambrósio

Treatment of retinal degenerative diseases: targeting neuroinflammation?

António Francisco Ambrósio (1, 2, 3)

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Retinal degenerative diseases, such as diabetic retinopathy, glaucoma and age-related macular degeneration (AMD), are leading causes of vision loss and blindness worldwide. These pathologies affect more than 150 million people and have no cure. Moreover, the treatments available are scarce, not always effective, and directed for the later stages of the disease, particularly in diabetic retinopathy and AMD. Taking these facts into account, there is an urgent need for better and more effective therapies.

In the last 15 years, accumulating evidence has shown that chronic inflammation plays an important role in the pathogenesis of retinal degenerative diseases. Evidence has also demonstrated that microglia, the immune cells of the central nervous system, are key elements in the inflammatory process. Therefore, targeting inflammation, and particularly microglial cells, might be a strategy to halt or at least to delay the progression of retinal degenerative diseases.

We have been identifying molecular players, such as nitric oxide, TNF and IL-1beta, which have a key role in blood-retinal barrier dysfunction, as well as in neural dysfunction and neurodegeneration in the retina, by using animal and in vitro models of diabetic retinopathy and glaucoma. Moreover, we have shown that by blocking adenosine A2A receptors or activating neuropeptide Y receptors, we have been able to inhibit microglia activation and protect retinal neurons. Some drugs already used in the market have pleiotropic effects and are also able to inhibit inflammation and microglia activation.

This group of results reinforces the evidence that inflammation has a key role in retinal dysfunction and that targeting microglia might be a promising therapeutic strategy to treat retinal degenerative diseases.

New insights on psychostimulant regulation of microglia activation

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Exposure to psychotropic stimulants has been primarily associated with damage to dopaminergic neuronal terminals and oxidative stress. However, it is now widely accepted that the interaction between neuronal and glial cells plays a critical role in the development of drug addiction. Accordingly, exposure to psychostimulants has been repeatedly shown to produce neuroinflammation. At the behavioural level, withdrawal from such drugs leads to symptoms that resemble the “sickness behaviour syndrome”, which is characteristic of primed microglia states and that is expected to increase the probability of relapse. Consequently, we hypothesise that the long-term adverse neuropsychiatric consequences of psychostimulant exposure may be due, at least in part, to an underlying modulation of microglia activity. Limiting this process may be relevant to control the addictive behaviour and reduce relapse. However, contrary to the common held view, our initial data revealed that methamphetamine (METH), a potent psychostimulant known to induce neuroinflammation in humans, cannot stimulate microglia in a cell-autonomous manner. To clarify how METH could modulate microglia signalling in vivo, we first tried to activate microglia through exposing different types of primary neuronal cells to METH. Surprisingly, independently of the METH dosing and exposure-time assayed, we could not promote such activation. We found, however, that METH could activate microglia via astrocytes. Conditioned media from astrocytes exposed to METH induced a pro-inflammatory phenotype in microglia, characterised by a significant increase in ROS production, iNOS expression and phagocytic activity. We further observed that in primary astrocytes METH exposure was not affecting the production of pro-inflammatory cytokines (IL-1β; IL-6 and TNF), but was promoting a massive release of glutamate. To gain mechanistic insight into astrocyte-induced microglia activation, we explored the pathways involved in astrocytic glutamate release. Previous studies have shown that TNF controls glutamate release from astrocytes. In accordance, we saw that METH-induced glutamate release was largely attenuated in TNF knockout astrocytes. We further demonstrated that METH and TNF regulated astrocytic glutamate release via mobilisation of Ca2+ from the endoplasmic reticulum to the cytosol in an IP3R-sensitive manner and via SNARE-dependent exocytosis. Confirming this mechanism, TNF knockout mice exposed to METH do not display neuroinflammation and when tested in the elevated plus maze do not present the classic METH-induced behaviour alteration. We anticipate that preventing METH-induced exacerbation of microglial activation will prove also beneficial in preventing relapse episodes. This knowledge can be easily translated into clinical applications, since there are readily available FDA-approved medications that can be used in abstinent patients.
Session III – Neuroinflammation

Andrea Cruz

Identification of actomyosin-based biomarkers relevant for microglia activation

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Neurological diseases are the result of progressive loss of neurons in the peripheral and central nervous system (PNS and CNS, respectively), and the major cause of cognition and motor impairment. The mechanisms underlying these diseases rely on a chronic activation of the innate immune system mediated by Microglia (MG) cells (at the CNS), and macrophages (at the PNS). MG cells represent up to 10% of adult brain cells and participate in the regulation of distinct physiological processes by controlling the repair, development, growth, and function of other brain cells. However, during disease progression, resting MG can polarize into more pro- or anti-inflammatory phenotypes. These functionally distinct MG have been associated to the emergence of different morphologies: resting MG are structurally ramified cells, while pro-inflammatory MG are more amoeboid and anti-inflammatory MG more bipolar. These biochemical and morphological alterations require profound cytoskeleton rearrangements, which are coordinated by actin and myosin based-complexes providing structural and mechanical support for cellular dynamics. Although, recent data show that physiological mechanical stimuli suppress inflammation whereas pathophysiological mechanical stimuli trigger inflammation, how MG cells convert cytoskeleton-based biomechanical stimuli into specific biochemical signatures remains poorly understood. In this context, our goal is to identify actomyosin-based biomarkers important for the regulation of MG pro-inflammatory and anti-inflammatory polarization. Our results indicate that, in resting MG, modulation of specific molecules from the actin and myosin pathway, via different pharmacological approaches, can trigger pro- or anti-inflammatory polarization.
Session III – Neuroinflammation

Marta Barbosa

Recovery of miR-146a levels through overexpression reverts astrocyte reactivity in cells from ALS mice and modulate astrocytic exosome cargo

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Amyotrophic Lateral Sclerosis (ALS) is a disease characterized by the loss of motor neurons (MNs) from the motor cortex and spinal cord, although it is still not clear where the disease begins and disseminates. In addition, glial cells are determinant for disease progression, and recent data from our group point out that astrocytes from cortical brain of mice pups (7 days) expressing the G93A mutation in human superoxide dismutase 1 (mSOD1) have an aberrant/reactive phenotype profile characterized by SOD1high/GFAPlow/S100Bhigh/Glutamate transporterslow/Ki-67high/vimentinhigh/miRNA-146alow (unpublished data). These astrocytes revealed neurotoxic properties when co-cultured with NSC34-like MNs. Such neurotoxic potential may be exerted by astrocyte-derived exosomes. Therefore, this work aimed to evaluate whether the recovery of the miR-146a expression (i) will impact on the astrocyte reactivity and (ii) on their exosome miRNA profiling.

For that, we used astrocytes isolated from the cortical brain of mSOD1 mice pups and cultured for 13 days. Cell were then modulated for miR-146a expression by using pre-miR-146a (mSOD1 astrocytespre-146a). Non-transgenic littermates (wt) were used as controls. Exosomes were isolated by differential ultracentrifugation. Isolated mSOD1 astrocytes showed increased levels of S100B and connexin-43 (Cx43) gene expression, as well as reduced ones GFAP mRNA and protein levels, thus corroborating their aberrancy. We identified decreased levels of miRNA-21 and unchanged alterations of miR-155 in these astrocytes, as compared with wt astrocytes. After successfully increase of miR-146a expression in mSOD1 astrocytespre-146a, we were able to observe a reduction on both miR-155 and miR-21 expression. In addition, in mSOD1 astrocytespre-146a we obtained a reduction of S100B and Cx43 mRNA levels, as well as increased levels of GFAP protein, thus reverting, at least in part, the aberrancy of mSOD1 astrocytes. Remarkably, astrocyte-derived exosomes showed to recapitulate the miRNA expression pattern of the modulated mSOD1 astrocytespre-146a, e.g. miR-155 downregulation and miR-146a upregulation relatively to their untreated counterparts.

Overall, our results highlight the influence of miR-146a upregulation in reversing astrocyte aberrant phenotype. Therefore, miR-146a may be a potential target to driven therapies. Furthermore, modulatory intervention in astrocytes was able to also trigger an increase of exosomal content in miR-146a, which may have a broader impact in preventing, halting or even reverting the harmful effects of astrocytes on MNs in ALS disease.

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Microglia homeostasis requires RhoA and its conditional ablation leads to phenotypes commonly associated with neurological disorders

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Microglia are the resident myeloid cells of the central nervous system and their functions are critical for brain functioning in health and disease. Here, using tissue-specific conditional gene targeting in mice we show an essential role for the small GTPase RhoA in regulating microglial homeostasis in the adult brain. We report that microglia-specific ablation of RhoA persistently disrupted the homeostasis of brain resident microglia and reduced their numbers in the brain parenchyma, ultimately leading to neuroinflammation, astrogliosis, angiogenesis, alterations in hippocampal synaptic protein expression and behavioral deficits. We also found that RhoA exerted its microglial homeostatic functions by sustaining C-terminal Src kinase (Csk) negative regulation of c-Src tyrosine kinase activity. Using a clinically relevant c-Src inhibitor in mice deficient for RhoA in microglia prevented the disruption of microglial homeostasis and inhibited neuroinflammation. Overall, our results revealed RhoA signaling as a major regulator of microglial function, highlighting an essential and primary role for microglia as gatekeepers of CNS physiology.